REMARKS

I. Status of the claims

Claims 26-83, 85, and 93-143 are pending. Claims 93, 98, 101, and 113 have been amended solely for clerical and grammatical purposes. Claims 1-25, 84, and 86-92 were previously canceled without prejudice or disclaimer. Claims 26-83 and 85 are withdrawn from consideration. Applicants reserve the right to file one or more continuing applications to the cancelled subject matter.

Applicants thank the Examiner for pointing out that claims 93 and 113 contain two step "(iv)"s, which they have amended appropriately.

i. The present application discloses a "repeatable" process for producing chromosomal fragments, contrary to the Examiner's assertion that the application discloses only spontaneous fragmentation via irradiation

Claims 93-126 have been rejected under 35 U.S.C. § 112, first paragraph, for alleged failure to comply with the enablement requirement. According to the Examiner, undue experimentation is placed upon the person of skill in the art to produce a recombinant chromosome with any chromosomal fragments, because the specification allegedly teaches only fragments from chromosomes 2, 14, and 22.

The Examiner states that the "specification does not disclose a repeatable process to obtain the SC20, W23 fragments and the 6-1 clone because it teaches the generation of the fragment by spontaneous fragmentation by irradiation." Still, the Examiner invites Applicants to "point to specific support in the specification by page and line number" if they feel that a "repeatable" process is so disclosed.

In light of the Examiner's invitation, Applicants take this opportunity to point out that the application indeed does provide a "repeatable" process for making the claimed recombinant chromosome. The application also emphasizes the "need to develop techniques which make it possible to freely process a chromosome" so that it may be stably retained in a cell (page 63, lines 9-12). This is because "an accidentally fragmented chromosome," such as

one that is generated spontaneously by irradiation, may be "unstable in a mouse, or may affect development of the mouse so that a human chromosome-introduced mouse itself cannot be generated" (page 62, lines 15-20). Thus, techniques are needed "that do not depend on accidentally obtained chromosomal fragments, but enables us to cleave a human chromosome at a desired site or to combine only a desired chromosomal fragment with another stable chromosome" (page 63, lines 18-22).

Applicants report that "a microcell prepared from the mouse-human hybrid cell may be irradiated with γ-rays such that the marked human chromosome is fragmented and transferred into a mouse A9 cell" (page 59, lines 3-6). But the irradiation method is by no means the only method of fragmenting a chromosome. Applicants state, for instance, that human chromosomes may be "modified by deletion, translocation, substitution and the like" (page 59, lines 14-16). In fact, Applicants disclose both the use of a "telomere-directed truncation" methodology, for cleaving chromosomes, and a loxP/Cre homologous recombination system to recombine the resultant fragments. For instance, see section A, "Modification of human chromosome 22" (page 64, line 17 to page 65, line 7), section B "Modification of human chromosome 2" (page 64, lines 9-24), and section C, "Modification of human chromosome 14 fragment SC20" (page 65, line 26 to page 66, line 12). These telomere-directed truncations and loxP/Cre translocations between chromosomes "enable the cloning of human chromosomal fragments with any size" (page 70, lines 8-12).

Applicants also point the Examiner to Examples 87-111 (pages 297-339) of the specification, which relate (i) the *site-directed* cleavage, not *irradiated* cleavage, of human chromosomes and (ii) the subsequent transformation of CHO cells with the fragments thus generated. For instance, Example 95 (page 310, line 5 to page 313, line 7), describes the "[C]leavage of chromosome #2 **at** an insertion site" (emphasis added; page 310, line 7) and that "the human chromosome #2 was cleaved **at** the CD8A locus in the homologous recombinant CD clone" (emphasis added; page 313, lines 1-2).

Thus, Applicants teach that this "invention provides a *universal system* for constructing human artificial chromosomes" (emphasis added; page 64, lines 5-6). In other

words, a key aspect of the present invention lies in providing a "repeatable" process for engineering chromosomes, which does not rely solely on the spontaneous chromosome fragmentation by irradiation. By the same token, the various techniques for fragmenting and manipulating chromosomes apply to *all* chromosomes and not only to those exemplified in the present application.

Applicants have met the Examiner's request to identify specific support in the specification "by page and line number" for the disclosed "repeatable process" and, for at least the reasons set forth in the preceding passage, Applicants respectfully request that the Examiner withdraw this rejection.

ii. Contrary to the Examiner's assertion, there is sufficient written description support in the specification for a chromosome 21 fragment

Claims 127-143 have been rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the written description requirement. According to the Examiner, the specification allegedly "fails to provide sufficient teachings or guidance with regard to other chromosome fragments, other than the exemplified chromosome #2 and #22 fragments" (Office Action at page 7) and that it "fails to provide a specific description of the chromosome 21 fragment." Office Action at page 10.

In reply to the Examiner's invitation to "point to a particular page and line number" for support for the "chromosome 21 fragment," Applicants respectfully direct the Examiner to page 66, line 20 to page 67, line 3, of the specification. There, Applicants relate that "it was found that human chromosome 21 (fragment) screened from a monochromosome hybrid cell library that has been constructed by the methods described in Tomizuka *et al.*'s report (Nature Genet., 16: 133, 1997) was also very stable in a chimeric mouse. That is, a chimeric mouse was produced from mouse ES cells, which retain chromosomal fragments containing most of human chromosome 21 (chimerism: 95 % or more). The retention rate of the chromosome in a brain cell, a hepatic cell, and a fibroblast derived from the tail of this chimeric mouse were all 95% or more. Hence, it is concluded that human chromosome 21 and fragments thereof can be used as a human artificial chromosome vector, as in the above SC20."

Furthermore, Applicants consider as part of their invention, "[A]n artificial chromosome vector, which comprises a centromere sequence derived from a human chromosome #14 or #21, and a recognition sequence for a site-directed recombination enzyme" (page 46, lines 15-18).

For at least these reasons, Applicants respectfully assert that there is sufficient written description for a fragment of human chromosome 21, and ask that the Examiner withdraw this rejection.

iii. The present specification enables the use of chromosome fragments from chromosome 21, as well as fragments chromosomes other than chromosomes 2, 14, and 22

Claims 93-126 have been rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the enablement requirement. According to the Examiner, the specification does not teach how to produce or use a recombinant chromosome that comprises a chromosome 21 fragment that contains a centromere and that, therefore, undue experimentation is placed upon the person of ordinary skill to make and use the claimed invention.

(a) There is no undue experimentation required to make and use the claimed invention because Applicants provide a written description for chromosome 21 and an enabling disclosure for manipulating chromosomal fragments

As outlined in the preceding subsection, the present application does indeed contemplate the use of fragments of chromosome 21. Applicants provide detailed technical guidance and numerous actual working examples (Examples 1-111) that show in precise detail how to make and use the presently claimed invention. Hence, Applicants teach how to perform a telomere-directed truncation of a chromosome and how to recombine or translocate the resultant chromosome fragment with other genetic material. That Applicants demonstrated these manipulations with, specifically, chromosomes 2, 14, and 22, does not negate the manipulation of other desired chromosomes.

Indeed, the determination that "undue experimentation" would have been needed to make and use the claimed invention is not a single, simple factual determination. Rather, it is a conclusion reached by weighing all factual considerations. *In re Wands*, 858 F.2d at 737, 8 USPQ2d at 1404. It is improper to conclude that a disclosure is not enabling based on an analysis of only one factor, while ignoring one or more of the others. The Examiner's analysis must consider all the evidence and any conclusion of non-enablement must be based on the evidence as a whole. 858 F.2d at 737, 740, 8 USPQ2d at 1404, 1407.

Applicants have shown, therefore, how to construct a recombinant chromosome with three different chromosomal fragments. It is not necessary that they provide experimental evidence for each embodiment of their invention. Applicants have provided a written description for chromosome 21 and an enabling disclosure for manipulating chromosomal fragments to practice the claimed invention. The person of skill in the art is unencumbered, therefore, and faces no undue experimentation to practice the claimed invention.

(b) The present claims do not require the recited chromosome fragments to function together nor do they preclude the co-existence of different genes on the same recombinant chromosome

The Examiner also alleges that "the specification fails to provide guidance with regard to the generation of recombinant chromosomes that have *any* two chromosomal fragments which do not encode antibody gene loci, and one of skill in the art would not know how to use such a recombinant chromosome" (emphasis added; Office Action at page 7). The Examiner states that "the breadth of the claim encompasses combinations of two proteins which do not function together, for example, an antibody locus and the amylase locus" (Office Action at page 7).

Applicants revert to pertinent language of claims 93 and 113, which require the recombinant chromosome to comprise "at least two chromosome fragments that had not been adjacently located in a natural chromosome or in a naturally occurring chromosome fragment." There is no functional mandate imposed on the recited chromosome fragments, nor any requirement that any two fragments "function together." It is entirely feasible to carry,

on the same recombinant chromosome, "at least two chromosome fragments" that encode different, unrelated genes.

In this respect, Applicants teach that "antibody," "T cell receptor," "histocompatibility antigen," and "dystrophin," for instance, are large (> 1 Mb) genes that are "[U]seful and interesting human genes which are desirably transferred into mice" (page 5, lines 11-19). Similarly, Applicants point out that many of the "causative genes" for human hereditary diseases and chromosomal aberrations, such as those that cause congenital deformities like in Down's syndrome, are typically located in "G bands" that are many megabases in size (page 5, line 29 to page 6, line 9), and these, likewise, would be suitable gene candidates for the present recombinant chromosome.

Applicants also note that the present invention seeks to "demonstrate successful transfer and expression in mice of uninterrupted foreign DNA fragments having a length of at least 1 Mb," an accomplishment which, prior to the present invention, had not been reported in the art (page 5, lines 7-10). An overall aim of the invention, therefore, is to provide a recombinant chromosome that is a hybrid of various chromosome fragments.

Furthermore, the application is replete with examples and experimental techniques for making the recited recombinant chromosome. This, when combined with the teachings related above regarding "useful genes," would govern any decision the skilled artisan may make about combining chromosomal fragments. Applicants do not believe, therefore, that any experimentation that the person of ordinary skill in the art may have to undertake in order to practice the claimed invention, would be considered "undue." Applicants respectfully request, therefore, that the Examiner withdraw this rejection.

iv. Claims 93, 94, 95, 98, 101, and 113 are not unclear

(a) *Claim 93 and claim 113: The qualification recited in claims 93 and 113, that the chromosome fragments "had not been" adjacently located, is clear and free from objection since the qualification informs the person of ordinary skill of the source of the fragments, not of their ultimate arrangement within the claimed recombinant chromosomes*

Claims 93 and 113 have been rejected under 35 U.S.C. § 112, second paragraph, because the Examiner finds unclear the phrase "had not been" in claim 93. According to the Examiner, the phrase lacks clarity because it "does not provide an indication of where the two fragments are with relation to each other. For example, the two chromosomal fragments could still not be adjacent to each other." Office Action at pages 12 and 13.

Applicants assert that they are not required to provide any indication in claim 93 or claim 113 as to the extant spatial relationship between the recited chromosomal fragments. The claimed recombinant chromosome requires five components, namely, (i) the centromere of human chromosome #14; (ii) two telomeres; (iii) at least one recognition sequence for a site-directed recombination enzyme; (iv) at least two chromosome fragments; and (v) a marker gene.

Thus, in a chromosome that comprises features "A-B-C-D-E," fragments "B" and "D" are not adjacent to one another. Accordingly, a recombinant chromosome that comprises those two particular chromosome fragments would meet the requirement recited in claim 93, subsection (iv). In this respect, fragments "B" and "D" may, or may not, be adjacent to one another in the claimed recombinant chromosome. The qualification that the chromosome fragments "*had not been* adjacently located in a natural chromosome or in a naturally occurring chromosome fragment" informs the skilled person only as to the *past* orientation of the fragments. Conversely, the qualification is clear in not prescribing the arrangement of the fragments within the recombinant chromosome; the claim is neutral on this point. Accordingly, the phrase "had not been" is transparent in its meaning, and Applicants therefore request that the Examiner withdraw these rejections.

(b) *Claim 94: claim 94 requires that a chromosomal fragment present in the recombinant chromosome must come from a region of chromosome 14 that does not flank the centromere of chromosome 14, or must come from another chromosome*

Claim 94 has been rejected under 35 U.S.C. § 112, second paragraph because the Examiner considers it to be unclear. The Examiner asks "is the chromosome fragment that is not naturally located adjacent to the human chromosome #14 fragment from another chromosome?"

Claim 94 further qualifies the claimed recombinant chromosome as comprising a chromosomal fragment that "is not naturally located adjacent to said human chromosome #14 fragment." Thus, one of the chromosomal fragments in the recombinant chromosome must come from either a region of chromosome 14 that does not flank the centromere of chromosome 14, or from another chromosome.

In this respect, there is no requirement in either claims 93 or 94 that the chromosomal fragments must be derived from the same chromosome. Indeed, claim 101 qualifies the recombinant chromosome as comprising "a human chromosome #14 fragment and a human chromosome #22 fragment."

Accordingly, Applicants believe that claim 94 is clear and that no amendment is necessary, and respectfully request that the Examiner withdraw this rejection.

(c) Claim 95: claim 95 recites a particular characteristic of SC20, namely that SC20 contains a centromere, which by itself is supported by the written description and so is clear and free from objection

Claim 95 has been rejected under 35 U.S.C. § 112, second paragraph because the Examiner does not know "if there are more than one centromere comprising portions of SC20."

Claim 95 requires qualifies the human chromosome #14 fragment of claim 94 to be "a centromere-comprising portion" of SC20. Applicants state that "human chromosome 14 fragment SC20, is retained almost 100% in a mouse and can be transmitted to progeny" and

that, therefore, "in this invention, SC20 is used as a human artificial chromosome vector" (page 65, line 27 to page 66, line 1). Applicants further experimentally deduced that the "SC20 fragment contains a human-derived centromere sequence" (page 234, lines 23-25). See, also, Example 68 at pages 232-246, which describes Applicants' experiments with SC20.

Thus, Applicants have provided much technical description regarding the structure and functional properties of SC20. Applicants do not believe, therefore, that it is necessary to amend claim 95 to qualify SC20 as containing only one or more centromere portions of SC20. The fact remains that the present application contains sufficient written description support for "a centromere-comprising portion of the chromosome fragment denoted as SC20," as presently recited. Applicants respectfully request, therefore, that the Examiner withdraw this rejection.

(d) *Claim 98 and claim 101: Applicants have amended claims 98 and 101 to better clarify and provide correct antecedent basis for the recited chromosome fragments*

Claims 98 and 101 have been rejected under 35 U.S.C. § 112, second paragraph because the Examiner states that "there is no reference to whether these fragments [either chromosome fragments 2 and 14, or 14 and 22] are the same fragments recited in part (iv) of claim 93."

Applicants thank the Examiner for suggesting how best to amend claims 98 and 101 (Office Action at pages 13 and 14) and have amended claims 98 and 101 appropriately. Those claims now clarify and provide appropriate antecedent support for the recited chromosome fragments. Accordingly, Applicants respectfully request that the Examiner withdraw this rejection.

v. The Examiner previously withdrew Tomizuka et al. as anticipatory art but now reapplies it against claim 93, but Tomizuka does not teach each and every limitation of that claim

Claim 93 is rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Tomizuka *et al.*, *Nature Genetics* 16:133-143, 1997 (of record).

To anticipate a claim under 35 U.S.C. § 102, a single prior art reference must disclose each element of the claimed invention. *See Scripps Clinic & Research Foundation v. Genentech, Inc.*, 927 F.2d 1565, 1576, 18 USPQ2d 1001, 1010 (Fed. Cir. 1991); *In re Bond*, 15 USPQ2d 1896 (Fed. Cir. 1991). Thus, Tomizuka *et al.* must teach a recombinant chromosome that comprises (i) the centromere of human chromosome #14; (ii) two telomere sequences; (iii) at least one recognition sequence for a site-directed recombination enzyme; (iv) at least two chromosome fragments that had not been adjacently located in a natural chromosome or in a naturally occurring chromosome fragment; and (v) a marker gene, wherein the recognition sequence for a site-directed recombination enzyme is located between said two chromosome fragments. Contrary to the Examiner's position, Tomizuka *et al.* does not teach each and every element of claim 93.

Applicants note that Examiner Ton withdrew from the October 23, 2002, Office Action, any rejection that was "not made of record" in the subsequent action of July 2, 2003. Accordingly, in the July 2nd Office Action, the Examiner's withdrew the rejection of the pending claims as allegedly anticipated by Tomizuka *et al.*, *Nature Genetics*\ 16:133-143, 1997.

Applicants further note that the Examiner's reasons for now reasserting Tomizuka against claim 93 are identical to those set forth in the withdrawn rejection. The only difference is that the Examiner now alleges that Tomizuka meets the "not adjacent" limitation of claim 93. Specifically, the only text of the anticipation rejection that differs from the October 23rd action is that which begins at page 15, line 18 ("Furthermore, the cited art ...") to page 16, line 10 ("...the claim reads on an entire chromosome with a loxP insertion").

Accordingly, Applicants take this opportunity to traverse the Examiner's rationale for rejecting claim 93 as set forth on pages 15, line 18 to page 16, line 10 of the Office Action.

According to the Examiner, chromosomal portions that are distal to one another "would not be considered 'adjacent' to each other." The Examiner relates that "two portions of a chromosome where a loxP site had been inserted between that were originally distal to each other would continue to be distal to each other. Thus, this meets the limitation of the claim."

Applicants appreciate the Examiner's exemplification, but stress that the presently claimed chromosome requires the presence of at least two distinct chromosomal *fragments*. Those two fragments are selected for inclusion in the recombinant chromosome on the basis that neither fragment existed next to the other in their unfragmented state. Thus, the claim requires the bringing together of at least two distinct and, previously-unconnected, chromosome fragments, into the claimed recombinant chromosome.

The Examiner now alleges, however, that Tomizuka's intact chromosome is identical to this recited chromosomal arrangement. According to the Examiner, any chromosome that comprises a loxP insertion site would anticipate claim 93 ("... the claim reads on an entire chromosome with a loxP insertion," Office Action at page 16). Clearly, this is not the case. The situation exemplified by the Examiner does not involve fragmented chromosomes and does not result in a composition that comprises these discreet structural elements.

Tomizuka also describes "very large DNA fragments," not only intact chromosomes (page 140, first column, fourth full paragraph, lines 1-3). Following the Examiner's own rationale, the ends of a large DNA fragment would be distal to one another and would remain distal if a loxP site was placed in the middle of the fragment. Accordingly, the distal ends of that fragment would not be "adjacent" to each other and, therefore, the claim limitation would be met. Thus, according to this reasoning, only *one* of Tomizuka's fragments is needed to meet the requirements set forth in claim 93. Yet, this does not meet the structural requirements of claim 93.

Claim 93 requires the recombinant chromosome to possess co-joined chromosomal *fragments*. The placement of a recognition site into Tomizuka's intact chromosome or fragment, as posited by the Examiner, does not implicate co-joined fragments, as prescribed.

In addition, claim 93 recites a recognition site *between* the aforementioned fragments. This arrangement does not pertain when a site is situated within a single chromosome or chromosome fragment, as posited by the Examiner.

In any event, Tomizuka does not teach the intentional insertion of a recognition sequence of a site-specific recombination enzyme into a desired site of a chromosome; hence, the reference cannot anticipate the subject matter of claim 93. On the other hand, Applicant's described method for preparing the present recombinant chromosome ensures that a recognition sequence is "positioned at desired site" of the recombinant chromosome. See, for instance, page 64, lines 27-29 and page 65, lines 19-22 of the specification. See also method claim 117.

While the claimed product thus includes a recognition site at a predetermined locus, a fragment according to Tomizuka certainly does not necessarily sport such a site at that locus. Yet the Examiner's rationale for anticipation turns on inherence, which requires this necessary result. For this reason, too, the rationale for rejection is faulty.

Given these distinctions, and the fact that the Examiner previously withdrew Tomizuka as anticipatory art, Applicants assert that claim 93 is not anticipated by Tomizuka, and respectfully request that the Examiner withdraw this rejection.

II. Conclusion

In view of the above remarks and amendments, it is respectfully submitted that this application is in condition for allowance. Early notice to that effect is earnestly solicited. The Examiner is invited to telephone the undersigned at the number listed below if the Examiner believes such would be helpful in advancing the application to issue.

Respectfully submitted,

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